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Soluble Transferrin Receptor in the Study of Fetal Erythropoietic Activity

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In order to evaluate fetal erythropoiesis we measured red blood cells, hemoglobin, hematocrit, serum transferrin receptor (sTfR), and iron status parameters in fetuses undergoing percutaneous umbilical blood sampling, and in normal newborns at term. We found high levels of sTfR in fetuses and newborns as compared with normal adults (3,149 ± 181 vs. 1,881 ± 137 ng/ml, $P < 0.00001$). Concentrations of sTfR correlate with gestational age and red blood cell numbers ($r = 0.441$, $P < 0.001$; $r = 0.366$, $P = 0.06$). sTfR concentrations do not show correlation with iron status parameters. The increased sTfR concentration is consistent with the fact that fetal life is characterized by cell proliferation and tissue growth. sTfR concentration correlates with gestational age and numbers of red blood cells, and can therefore be considered a good indicator of fetal erythropoiesis. It is conceivable that, during intrauterine life, sTfR expression is independent from iron status. sTfR determination will help in reaching a better understanding of some aspects of fetal physiology, and will help elucidate the physiopathology of fetal hematological diseases.

Key words: fetus, cordocentesis, erythropoiesis, serum transferrin receptor, iron status

INTRODUCTION

In recent years immunoassays have been set up to measure the circulating form of transferrin receptor called "serum transferrin receptor" (sTfR). sTfR, being directly related to the number of erythroid precursors, provides a reliable, quantitative measurement of erythropoietic activity [1].

Since cordocentesis has been established as a safe means of access to fetal circulation [2], erythropoiesis during intrauterine life has been investigated by evaluating different hematological parameters [3–5]: hemoglobin, numbers of red blood cells, hematocrit erythroblasts, reticulocytes, and erythropoietin.

In this study we investigated erythropoietic activity in 43 normal fetuses at different gestational weeks, and in the cord blood of 20 normal full-term newborns, by evaluating hemoglobin, number of red blood cells, hematocrit, sTfR, and iron status parameters.

MATERIALS AND METHODS

Forty-three fetuses undergoing percutaneous umbilical blood sampling for prenatal diagnosis (of infections, hemoglobinopathies, rapid fetal karyotyping, or suspected isoimmunization) and subsequently found unaffected by the investigated condition were retrospectively considered as normal. Their gestational age ranged from 19–34 weeks.

Twenty cord-blood samples from full-term normal newborns from normal pregnancies were also studied. Blood counts were determined by a cell counter (TOA

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TABLE I. Hematological Parameters, sTfR Concentration, and Iron Status

Case	Week	Hb (g/dl)	RBC ($n \times 10^{12}$/l)	Ht (%)	sTfR (ng/ml)	Ferritin (ng/ml)	Iron (μg/dl)	TIBC (μg/dl)	Sat (%)
1	23	11.4	2.96	33.4	3,349.0				
2	20	11.4	3.03	33.8	2,842.0	4	54	201	26.8
3	24	11.2	2.74	32.0	2,130.0				
4	20	11.5	2.70	32.3	2,538.0				
5	35	12.6	3.50	37.6	3,869.0				
6	20	11.6	2.89	34.0	1,902.0	7	66	154	42.8
7	20	10.8	2.64	30.3	1,983.0	7	42	228	18.4
8	21	10.1	2.29	30.1	2,299.0				
9	27	10.3	3.46	38.2	2,910.0	12			
10	29	12.0	2.86	33.2	2,447.5	57.5			
11	32	13.7	3.41	36.7	4,478.6	48.9			
12	34	14.5	3.88	40.1	3,258.3	22.8	70	302	23.1
13	33	15.0	4.15	42.8	3,569.1	85.1	—		
14	34	10.5	2.87	29.0	2,029.0	21	62	301	20.6
15	22	14.0	3.19	37.8	4,275.0				
16	32	13.3	3.59	40.0	3,169.0	11	69	406	17
17	20	10.2	2.38	29.1	1,723.0	70			
18	20	11.1	2.88	32.6	6,053.0	37			
19	34	17.4	4.54	48.8	4,477.8	60			
20	33	17.1	4.69	48.2	2,842.2	56			
21	26	12.1	3.23	35.8	3,224.4	31	56	250	22.4
22	21	14.6	3.31	40.8	2,157.7	26	65	181	35.9
23	19	12.0	2.62	33.6	2,202.7	51	65	182	35.7
24	26	12.4	3.13	35.1	1,137.0	79	113	357	31.6
25	22	10.0	2.54	28.9	1,400.5	36	58	202	28.7
26	21	11.7	2.82	32.3	3,009.2	22	49	196	25
27	27	12.7	3.28	36.2	2,681.4	29	67	194	34.5
28	23	13.4	3.45	37.9	2,627.9	37	49	189	25.9
29	21	10.2	2.49	28.8	6,226.5	27	47	189	24.8
30	22	12.0	2.90	33.7	2,290.9	50	79	249	31.7
31	29	13.4	3.56	35.9	1,750.0				
32	35	13.5	3.52	37.6	3,131.7	26	78	265	29.4
33	28	13.3	3.80	39.4	5,003.3	39	78	251	31
34	22	11.8	2.95	34.0	4,024.1	20			
35	34	11.9	3.33	34.8	4,575.5	200			
36	19	11.9	2.68	32.7	4,202.3				
37	23	11.5	2.71	31.8	3,606.9				
38	33	15.0	4.22	40.7	3,166.1				
39	22	12.5	3.09	36.5	1,781.2	43.2	48	224	21.4
40	28	12.5	3.45	35.1	2,070.2				
41	32	14.2	3.52	41.20	1,986.9	27.5	73	292	25
42	34	14.7	3.98	38.7	1,633.7	132.2			
43	21	11.4	2.75	31.0	1,618.9	25			
44	39	17.9	4.76	54.4	3,986.0	294	122	270	45.2
45	39	15.7	4.41	54.1	7,229.0	287	228	260	87.6
46	39	15.8	4.32	47.5	5,105.1	43.3	103	322	31.9
47	40	14.0	3.88	45.7	4,684.8	138.5	141	292	48.3
48	40	16.1	4.59	51.6	2,315.7	117.8	105	247	42.5
49	40	15.7	4.34	51.2	5,736.2	71.3	195	230	84.7
50	40	17.4	4.74	54.2	5,410.0	135.7	190	284	66.9
51	40	15.8	4.45	45.6	4,357.1	56.8	184	309	59.5
52	40	14.2	4.05	43.8	2,332.3	243.4	174	289	60.2
53	40	15.3	4.24	46.6	6,395.3	31.1	173	291	59.4
54	39	14.5	4.11	43.4	2,971.5	153.9	248	287	86.4
55	40	18.0	4.73	52.2	3,886.6				
56	39	12.2	3.47	36.1	2,533.1				
57	40	14.8	4.15	44.1	2,807.0	215.7	176	347	50.7
58	40	14.8	4.03	44.4	6,000.8				
59	39	16.8	4.54	51.8	4,474.5				
60	39	16.8	4.48	53.3	3,265.0	356.9	208	288	72.2
61	39	15.0	4.83	46.2	5,554.5	177.9	136	330	41.2
62	40	16.6	4.63	49.7	4,836.5	174	202	226	89.3
63	39	15.5	4.15	46.3	4,881.2	130			

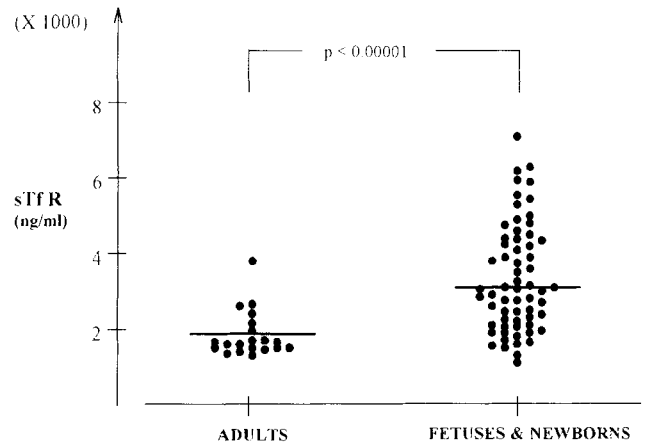


Fig. 1. sTfR concentration distribution between adults and fetuses and newborns.

NE-8000, Toa Medical Electronics Co., Ltd., Kobe, Japan), and sTfR was determined by an immunoassay (Quantikine™ Human Transferrin Receptor, R&D Systems Inc., Minneapolis, MN).

sTfR was determined in a group of healthy blood donors (10 males and 10 females, age range 20–60 years) in order to obtain adult normal reference values. Ferritin was determined by an enzyme immunoassay (Ramco Laboratories, Inc.) in 32 fetuses and in 16 full-term newborns. Iron and total iron binding capacity (TIBC) were determined in 20 fetuses and in 15 full-term newborns, according to standard colorimetric methods with an automatic chemical analyzer (Dimension® ES, Du Pont Medical Products, Wilmington, DE).

The protocol was approved by the S. Paolo Institute Board, and informed consent was obtained from all pregnant women undergoing the procedure of umbilical blood sampling. A computer-assisted regression analysis was used to evaluate the relationship between sTfR, gestational age, and hematological and iron status parameters. As sTfR does not show a normal distribution, calculations were performed after logarithmic transformation. Residuals were calculated for age-related variables, and correlations between residuals were calculated in order to eliminate age effect. The different sTfR distribution between normal adults and fetuses was studied with Mann-Whitney test.

Table I summarizes the studied parameters of fetuses and cord blood.

RESULTS

In normal adults, sTfR level was 1,881 ± 137 ng/ml, expressed as geometric mean ± standard error, whereas in the group of fetuses and newborns this was 3,149 ± 181 ng/ml. The difference was highly significant (Mann-Whitney test, $P < 0.00001$). The distribution is shown in Figure 1.

As expected, numbers of red blood cells, hemoglobin, and hematocrit correlated significantly with gestational age: $r = 0.874$, $P < 0.0001$; $r = 0.775$, $P < 0.0001$; $r = 0.81$, $P < 0.0001$, respectively. As these are dependent variables, we used as unique variable the number of red blood cells, as it showed the best correlation.

sTfR increased and correlated with gestational age ($r = 0.441$, $P < 0.001$); sTfR correlated also with red blood cells (Fig. 2), and sTfR residuals correlated with red blood cell residuals ($r = 0.366$, $P = 0.06$) (Fig. 3).

Iron, TIBC, and transferrin saturation increased and correlated exponentially with gestational age: $r = 0.851$, $P < 0.00001$; $r = 0.676$, $P < 0.0001$; $r = 0.658$, $P < 0.0001$, respectively. Ferritin, as previously shown [18], increased and correlated with gestational age ($r = 0.606$, $P < 0.00001$), and correlated also with iron (residual correlation: $r = 0.514$, $P < 0.001$). Residual correlations between sTfR and iron status parameters were not significant.

DISCUSSION

Transferrin receptor (TfR), which transfers iron from plasma (where it circulates bound to transferrin) to cells, is widely distributed in all tissues, but predominates in erythroid marrow, placenta, and liver [6].

The most important regulators of density of TfR on cells are: cell cycle, erythropoietin stimulation, and iron status [7–9].

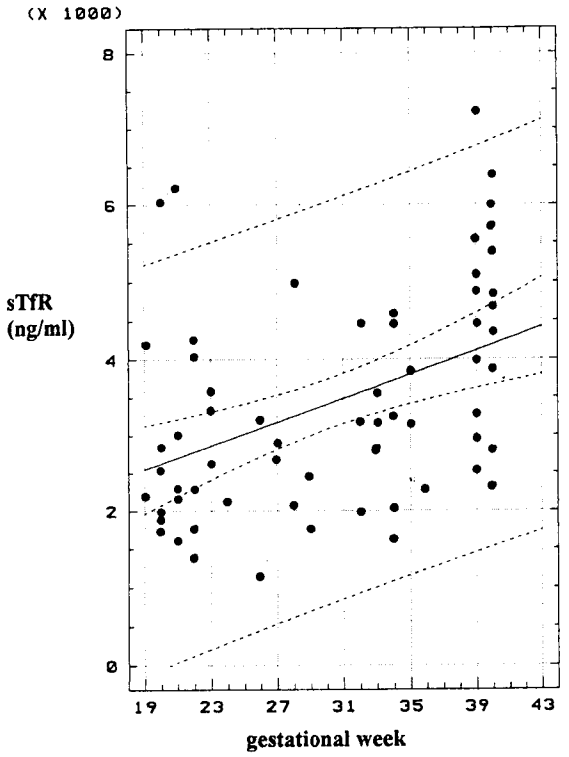
Soluble transferrin receptor (sTfR) is produced by the cleavage of the extracellular portion of the membrane-bound receptors. sTfR constitutes a constant fraction of tissue receptors, and its main source is erythroid marrow [10,11]. sTfR concentration is a quantitative measurement of erythron activity, strictly correlated with ferrokinetic measurements [12].

We have found that throughout intrauterine life sTfR values are higher than in normal adults (Fig. 1). Since iron is required to synthesize enzymes which are essential for the production of new cells [13], the high sTfR level found in this phase of life could partly reflect global fetal growth.

The correlation found between sTfR and, respectively, gestational age and numbers of red blood cells (Figs. 2, 3) suggests that in fetuses, as in adults, the erythroid marrow is a main source of sTfR and that its measurement can be considered a good indicator of expanding fetal erythroid tissue.

It is known that the placenta has both ferritin and transferrin receptors [14,15] and is actively involved in the transfer of iron from mother to fetus [16].

The fetal iron-status balance seems to be shifted towards iron uptake rather than to its utilization, as sug-



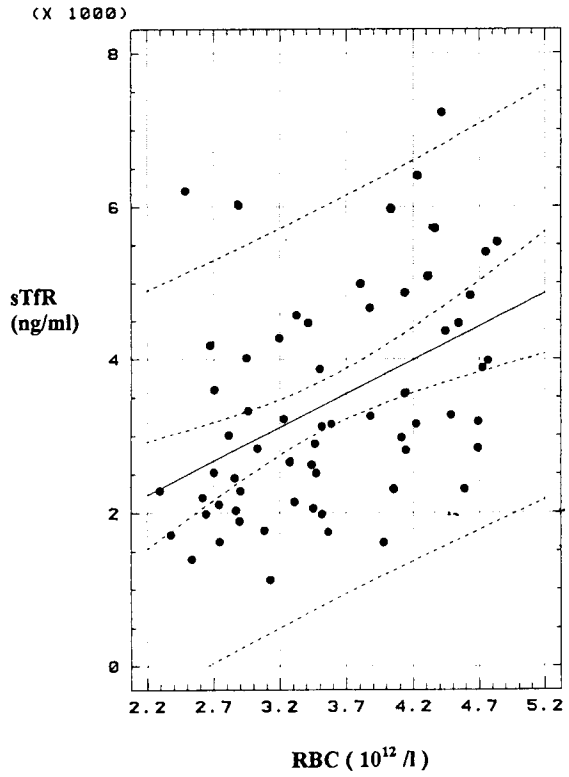
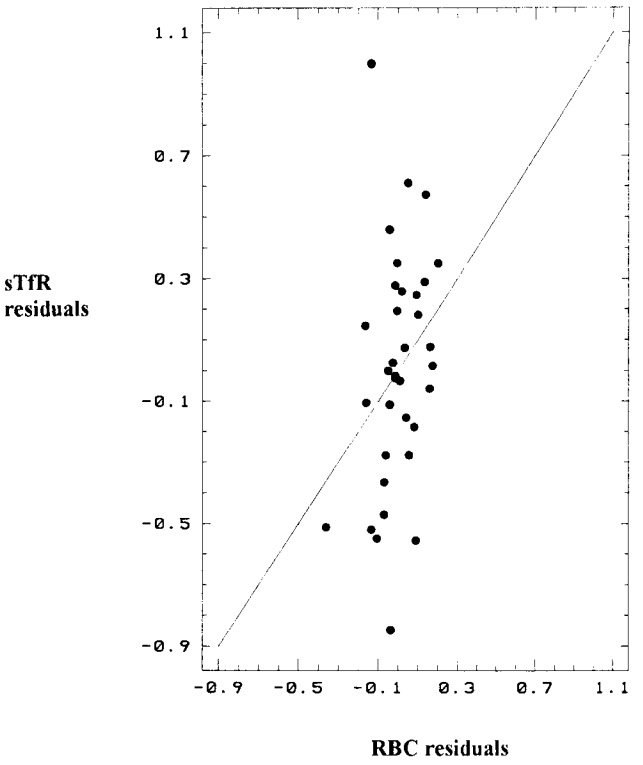


Fig. 2. sTfR concentration distribution vs. gestational week and red blood cell counts (mean and individual 95% confidence intervals).



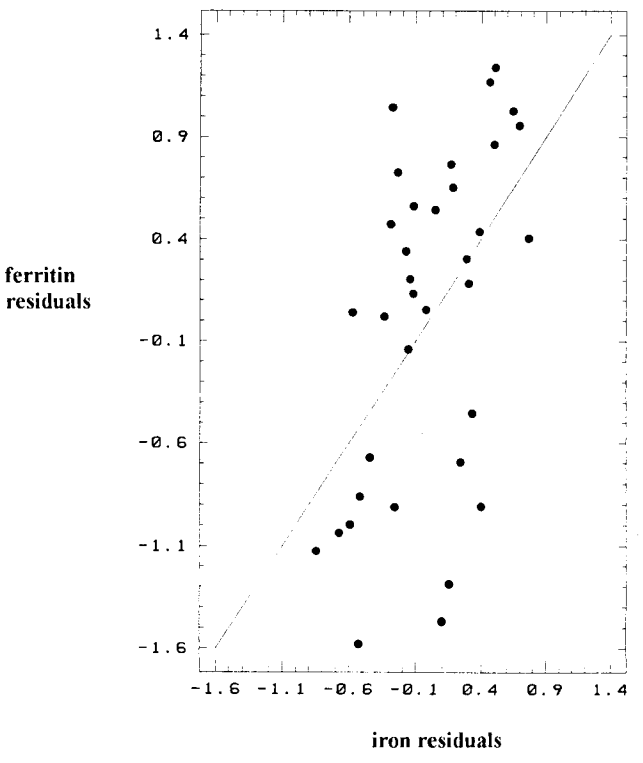


Fig. 3. sTfR residuals vs. red blood cell (RBC) residuals, and ferritin residuals vs. iron residuals.

gested by the increase with gestational age of plasma [17] and red cell ferritin [18] and by the high transferrin saturation found in the cord blood of healthy full-term infants [19]. Our data, showing increase of iron status throughout the gestational period, substantiate this hypothesis.

Iron status is a determinant of TfR expression via regulatory proteins, enhancing or diminishing the stability of TfR mRNA [20], and therefore fetal iron status could partly interfere with the density of TfR on fetal erythroblasts. However, experiments with developing chicken embryos have demonstrated a hyperexpression of the TfR gene which is not influenced by iron status and is regulated by other mechanisms [21]. The lack of correlation between sTfR and iron-status parameters suggests that in developing human beings, iron status is not a determinant for sTfR expression, which is consistent with reported hyperexpression of the TfR gene in differentiating erythroid cells of chicken embryos.

We conclude that sTfR determination in prenatal life is an important tool for measuring erythropoiesis; it is an interesting means for investigating some aspects of fetal physiology, and it merits clinical application in investigating certain fetal pathological conditions, e.g., intrauterine growth retardation [22].

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